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Contents

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■ IMMUNOLOGY

Histones and Genomic Stability: The Ties That Bind Broken Ends

Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, Reina-San-Martin B, Coppola V, Meffre E, Difilippantonio MJ, Redon C, Pilch DR, Olaru A, Eckhaus M, Camerini-Otero RD, Tessarollo L, Livak F, Manova K, Bonner WM, Nussenzweig MC, and Nussenzweig A. Genomic instability in mice lacking histone H2AX. *Science* 296: 922-7, 2002.

Genomic instability is a hallmark of cancer. Human hematopoietic malignancies are characterized by reciprocal translocations that juxtapose oncogenes and antigen receptor loci or that create oncogenic fusion proteins. Human solid tumors commonly contain an even more complex karyotype, consisting of chromosomal gains, losses, or gene amplifications. While the mechanisms leading to gross chromosomal rearrangements remain to be elucidated, studies in tissue culture cells have shown that DNA double-strand breaks (DSBs) may be the initiating lesion that triggers these events.

DNA DSBs arise in response to external genotoxic agents, as well as during replication and during programmed rearrangements in lymphocytes and germ cells. To counteract the deleterious consequences of DSBs, mammalian cells have evolved at least two distinct DNA repair pathways: homologous recombination (HR) and nonhomologous end joining (NHEJ). HR repairs DSBs by using an intact copy (homolog or sister chromatid) of the broken chromosome as a template, whereas NHEJ joins the two ends directly with little or no requirement for sequence homology. Loss of either HR or NHEJ promotes genomic

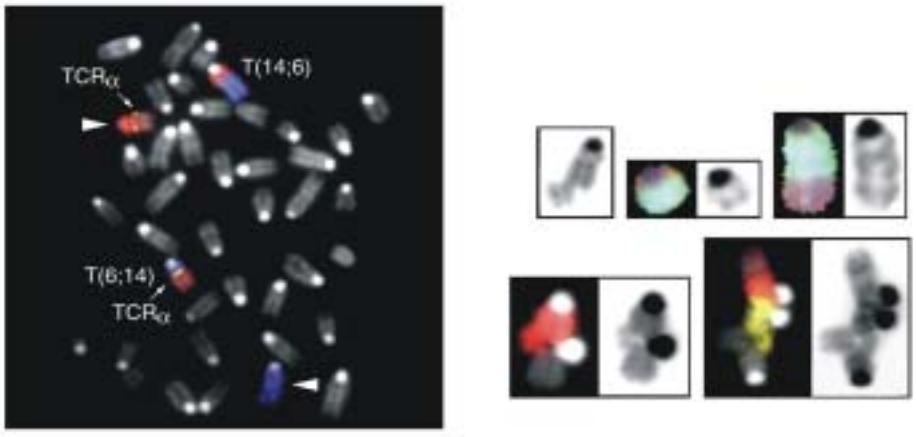

Figure 1. *Left:* Example of a reciprocal translocation in mouse H2AX$^{-/-}$ T cells in which the break point was near the T-cell receptor α locus (TCRα; arrows). Chromosome 14 (red) and 6 (blue) painting probes were used for fluorescence in situ hybridization (FISH) analysis together with a TCRα bacterial artificial chromosome probe. Arrowheads indicate normal chromosomes. *Right:* Typical chromosome aberrations of H2AX$^{-/-}$ T cells analyzed by DAPI (4',6 diamidino-2-phenylindole), spectral karyotype analysis, or FISH. Twenty-one percent of H2AX$^{-/-}$ T cells exhibited such chromosomal aberrations, which were not found in wild-type T cells.

instability and predisposition to cancer, suggesting that aberrant DNA repair pathways contribute to tumorigenesis.

Mammalian DNA is complexed with histones to form chromatin, and this higher order chromatin structure presents a barrier to the recognition and repair of DNA damage. Recently, William Bonner, Ph.D. (Laboratory of Molecular Pharmacology) discovered that a histone H2A variant, termed histone H2AX, becomes phosphorylated within seconds after induction of DSBs by exogenous agents (Rogakou EP, et al., *J Biol Chem* 273: 5858-68, 1998). Phosphorylated H2AX (termed γ-H2AX) appears to be a general response to DSBs, because γ-H2AX has also been detected at sites of physiological breaks in lymphocytes (Chen HT, et al., *Science* 290: 1962-4, 2000; Petersen S, et al., *Nature* 414: 660-5, 2001) and in germ cells (Mahadevaiah SK, et al., *Nat Genet* 27: 271-6, 2001) and is induced as a result of DNA fragmentation during apoptosis (Rogakou EP, et al., *J Biol Chem* 275: 9390-5, 2000). Covalent modifications of histones—including phosphorylation, acetylation, and methylation—have been proposed to form a code that is "read" by cellular machinery to facilitate downstream signaling events. Consistent with this model, several proteins implicated in HR and NHEJ form immunofluorescent foci in the nuclei of damaged cells that colocalize with foci containing γ-H2AX, which seems to mark the actual site of DNA damage (Paull TT, et al., *Curr Biol* 10: 886-95, 2000) even though the precise relationship between repair foci and DNA damage detection, signaling, and repair is not understood.

To help clarify the physiological role of H2AX in mammalian cells, a targeted disruption of mouse H2AX was produced (Celeste A, et al., *Science* 296: 922-7, 2002). $H2AX^{-/-}$ mice were viable but growth retarded and immune deficient, and the males were infertile. These phenotypes were associated with chromosomal instability, DNA repair defects, and impaired recruitment of various DNA repair factors into radiation-induced foci. For example, detailed analysis of T cells from $H2AX^{-/-}$ mice revealed abnormally high levels of random translocations and complex rearrangements, including reciprocal translocations in which the T-cell receptor α locus was proximal to the break point (Figure 1). Moreover, analysis of spermatocytes revealed that associated with the male-specific infertility was a high frequency of $H2AX^{-/-}$ nuclei in which X and Y chromosomes failed to pair, were fragmented, or were fused with autosomes. It appears that chromatin organization facilitated by γ-H2AX is needed to prevent the premature separation of broken DNA ends during replication, in response to irradiation, and during meitotic recombination. In addition, these results indicate that H2AX has a role in modulating DNA repair via HR as well as NHEJ.

Hereditary diseases affecting the cellular response to DSBs include ataxia telangiectasia, Nijmegen breakage syndrome, and Bloom's syndrome. The hallmarks of these disorders are growth defects, immunodeficiency, hypogonadism, sensitivity to specific DNA-damaging agents, chromosomal fragility, and predisposition to cancer. $H2AX^{-/-}$ mice share many of the pleotropic features of these "chromosomal instability" syndromes. $H2AX^{-/-}$ mice are small, exhibit male-specific sterility, and have reduced levels of secondary immunoglobulin isotypes, and their primary cells exhibit high levels of chromosomal abnormalities. Because of the role of H2AX in suppressing genomic instability and its predicted function as a tumor suppressor, it will be important to determine whether mutations in H2AX are found among humans with similar clinical features.

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Selenium and Health: Is Selenium a Magic Bullet?

Moustafa ME, Carlson BA, El-Saadani MA, Kryukov GV, Sun Q-A, Harney JA, Hill KE, Combs GF, Feigenbaum L, Mansur DB, Burk RF, Berry MJ, Diamond AM, Lee BJ, Gladyshev VN, and Hatfield DL. Selective inhibition of selenocysteine tRNA maturation and selenoprotein synthesis in transgenic mice expressing isopentenyladenosine-deficient selenocysteine tRNA. *Mol Cell Biol* 21: 3840-52, 2001. See also the cover of *Mol Cell Biol* 21: 2001.

Hatfield DL and Gladyshev VN. How selenium has altered our understanding of the genetic code. *Mol Cell Biol* 22: 3565-76, 2002.

Kumaraswamy E, Carlson BA, Morgan F, Miyoshi K, Robinson G, Su D, Wang S, Southon E, Tessarollo L, Lee BJ, Gladyshev V, Hennighausen L, and Hatfield DL. Selective removal of the selenocysteine tRNA gene (*Trsp*) in mouse mammary epithelium. *Mol Cell Biol* in press.

Selenium is an essential micronutrient in the diet of humans and other mammals. This element has been touted to be an important cancer chemopreventive agent, inhibit viral expression, delay the progression of AIDS in HIV-infected patients, prevent heart disease and other cardiovascular and muscle disorders, slow the aging process, and have roles in mammalian development, male reproduction, and immune function. The molecular biology of selenium metabolism in relation to human health has been reviewed in *Selenium: Its Molecular Biology and Role in Human Health* (Hatfield D, editor. Norwood, MA: Kluwer Academic Publishers, 2001). As more of the molecular biology of the biomedical effects of selenium is elucidated, selenium does indeed seem to possess multiple beneficial properties in health, raising the question "Is selenium a magic bullet?"

The means by which this element promotes better health is just beginning to be understood. Humans have approximately two dozen selenium-containing proteins (Vadim Gladyshev, Ph.D., University of Nebraska, personal communication), and many of these selenoproteins are most certainly responsible for the vast health benefits associated with dietary selenium. One such protein that Alan Diamond, Ph.D. (University of Illinois at Chicago), Dr. Gladyshev, and I have been studying, designated Sep15, has many properties consistent with it functioning in decreasing cancer incidence. A major focus of our laboratory, the Molecular Biology of Selenium Section (MBSS), is to devise mouse models for determining the role of selenoproteins in health and development. Such model systems are designed with the knowledge that selenium is incorporated into protein as the amino acid selenocysteine (Sec) through its tRNA. Sec tRNA is the only known tRNA that controls the expression of an entire class of proteins (the selenoproteins). Thus, altering the expression of this tRNA can perturb selenoprotein synthesis, providing an important tool to regulate the expression of individual members—as well as all members—within this protein group.

The genetic codeword UGA dictates the cessation of protein synthesis. Recently, however, this codon was recognized to also code for Sec, making Sec the 21st amino acid. Much of the machinery used for inserting Sec into a nascent polypeptide is unique to this amino acid. For example, the 20 common amino acids in the genetic code share the same elongation factor for their incorporation into protein, but Sec has its own elongation factor, EFsec. In addition, all selenoprotein mRNAs in eukaryotes have a specific stem-loop structure in their 3'-untranslated region that is recognized by the protein factor SPS2. SPS2 binds to the stem-loop structure, and in concert with the Sec elongation factor and Sec tRNA, donates Sec to the growing polypeptide chain in response to UGA in selenoprotein mRNAs (Figure 1). The stem-loop structure dictates that UGA will serve as a Sec codon and not as a stop codon. Indeed, compared with the other 20 amino acids in the genetic code, nature has invested considerable effort in evolving the specific machinery for incorporating Sec into protein.

The MBSS has devised various mouse model systems to examine the role of selenoproteins in health. Because the knockout of the Sec tRNA gene is embryonic lethal, *loxP/Cre* technology was used

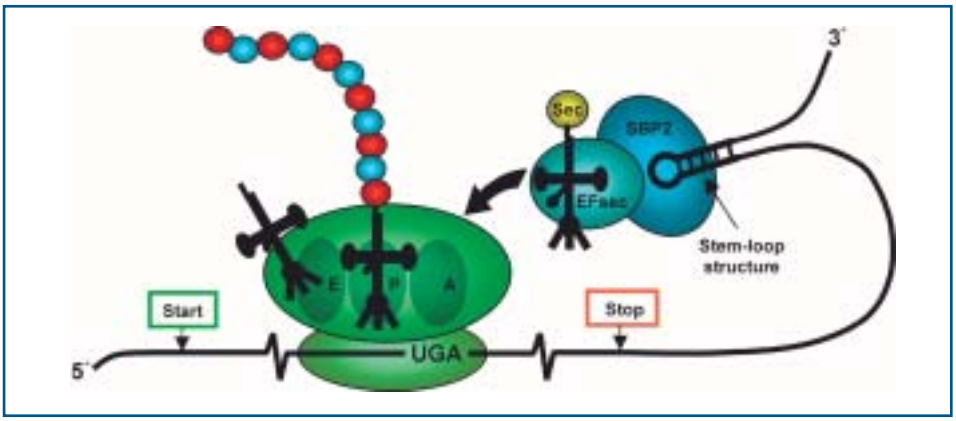

Figure 1. Mechanism of selenocysteine (Sec) insertion into protein in eukaryotes. Sec tRNA is complexed with its elongation factor (EFsec), which binds with SBP2, the protein factor that recognizes the stem-loop structure in the 3'-untranslated region of selenoprotein mRNA that is now ready for donation of Sec to the ribosomal A site. The growing peptide is depicted as alternating red and blue balls attached to a tRNA at the ribosomal P site. The E site represents the ribosomal exit site. mRNA is attached to the small ribosomal subunit and is depicted as a long, dark line with its 5' and 3' ends, start and stop codons, stem-loop structure, and the UGA codon for decoding Sec tRNA. (Reviewed in Hatfield DL and Gladyshev VN. *Mol Cell Biol* 22: 3565-76, 2002.)

to generate a conditional knockout of this gene in mouse mammary, liver, or prostate tissues. The Sec tRNA gene was reduced by 70% to 80% in mammary tissue and nearly 100% in liver or prostate. Thus, expression of selenoproteins can be specifically altered in a variety of tissues, providing a means of assessing their biological roles in decreasing the incidence of cancer (e.g., in preventing breast, liver, or prostate cancer). On the basis of results from an earlier human clinical trial suggesting that selenium protects against prostate cancer, a new NCI trial was recently initiated to examine the role of selenium and vitamin E in preventing prostate cancer (http://cancer.gov/select). The means by which selenium prevents prostate cancer and whether selenoproteins play a role in this process are still subject to debate. By determining the incidence and time of expression of prostate cancer in male mice and breast cancer in female mice that encode the knockout of the Sec tRNA gene and a cancer driver gene targeting these tissues (in collaboration with Jeffrey Green, M.D., Laboratory of Cell Regulation and Carcinogenesis), we can assess the ability of selenoproteins to protect against specific cancers.

The MBSS has also generated a mouse line that carries mutant Sec tRNA transgenes that produce Sec tRNA lacking the highly modified base isopentenyladenosine in its anticodon loop. The mutant mice manifest reduced translation of numerous selenoproteins in a protein- and tissue-specific manner. This study is the first example of mice engineered to produce functional tRNA transgenes. The response of these selenoprotein-deficient mice to a variety of stress conditions—such as viral infection (in collaboration with Melinda Beck, Ph.D., University of North Carolina, Chapel Hill), exercise-induced muscle adaptation following synergist ablation (in collaboration with Dr. Diamond and Karyn Esser, Ph.D., University of Illinois at Chicago), and specific cancer driver genes while the animals are maintained on selenium-deficient diets (in collaboration with Dr. Gladyshev) or selenium-sufficient diets (in collaboration with Dr. Diamond)—are expected to provide further insights into the roles of selenoproteins in health, including their ability to serve as anticarcinogenic agents.

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■ STRUCTURAL BIOLOGY

HIV in Chains: Characterization of the Multivalent Binding of the Anti-HIV Protein Cyanovirin-N with Branched Oligomannosides of gp120

Shenoy SR, Barrientos LG, Ratner DM, O'Keefe BR, Seeberger PH, Gronenborn AM, and Boyd MR. Multisite and multivalent binding between cyanovirin-N and branched oligomannosides: calorimetric and NMR characterization. *Chem Biol* 9: 1109-18, 2002.

The HIV envelope glycoprotein gp120 is heavily glycosylated, and carbohydrates represent approximately half the molecular weight of the glycoprotein. The architectures of the variable and conserved regions of gp120 are spatially and chemically different: highly processed oligosaccharides (complex type) are found near the variable domains, and less processed oligosaccharides (high-mannose type) occupy the conserved regions. The canyon theory for gp120-receptor binding predicts that proper topological discrimination of these chemically different regions is essential for effective neutralization of gp120.

Cyanovirin-N (CV-N), an 11-KDa protein originally purified from an extract of the cyanobacterium *Nostoc ellipsosporum*, binds tightly to high-mannose oligosaccharides on gp120 and gp41. In this manner, CV-N is able to inactivate a broad spectrum of HIV-1, HIV-2, and simian immunodeficiency virus (SIV) strains. The lectin-like anti-HIV activity of CV-N is at least 1,000-fold more potent (EC_{50} = 1 ng/ml) than the reported antiviral activities of other lectins (EC_{50} = 1–2 µg/ml), and its oligosaccharide-binding specificity is also unprecedented. CV-N has been shown to differentiate among high-mannose oligosaccharides, preferring to bind the larger, branched oligomannose sugar structures (Man-8 and Man-9) over the smaller ones (Man-5, Man-6, and Man-7). Only Man-8 and Man-9 significantly inhibit the CV-N–gp120 interaction and interfere with CV-N's ability to block gp120-CD4–mediated fusion. However, defining the exact mechanism by which CV-N binds Man-8 and Man-9 has been difficult because the CV-N complexes of these two sugars aggregate and precipitate.

To circumvent problems due to aggregation, a series of synthetic oligomannoside substructures of Man-9 was designed that could be used in structural and thermodynamic binding studies. Recent progress in solid-phase synthesis and solution-phase assembly of oligosaccharides has allowed the production of a branched nonamannoside, the closest synthetic homolog of Man-9 yet to be studied. The nonamannoside lacks the chitobiose unit at its reducing end but maintains the internal branched construction of Man-9 and, more important, the three mannosyl

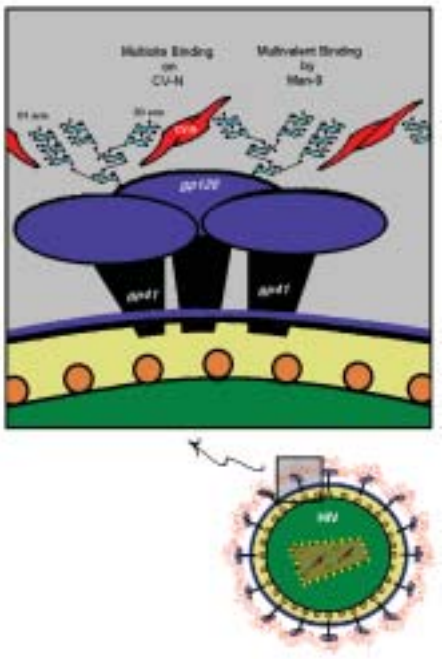

Figure 1. *Bottom:* The envelope of the HIV virus is coated with glycoproteins gp120 and gp41. The antiviral protein cyanovirin-N (CV-N) targets high-mannose oligosaccharides on these glycoproteins, cross-linking them and preventing receptor-mediated fusion of HIV with the host cell. *Top:* Schematic representation of multisite, multivalent interaction between oligomannose-9 (Man-9) and CV-N. The two oligosaccharide-binding pockets on CV-N can each accommodate α1→2-linked mannose structures of Man-9. The unhindered presentation of α1→2-linked mannoses on the D1 and D3 arms allows a single Man-9 to bind two molecules of CV-N. The overall simplified stoichiometry of the interaction is 1:1.

"arms" of the oligosaccharide. Smaller substructures of the nonamannoside, the hexameric and trimeric cores, and the linear trimeric arm (D1 arm) were also synthesized and studied. The binding of these oligosaccharides to CV-N was qualitatively assessed by nuclear magnetic resonance (NMR). The information obtained from these structural studies was used in conjunction with isothermal titration calorimetry experiments to yield a thorough quantitative picture of the interaction between CV-N and Man-9.

NMR titration experiments with the synthetic linear trimannoside, hexamannoside, and nonamannoside indicated that these oligosaccharides interact with CV-N at one or two regions of the protein. Particularly for the α1→2-linked linear trimannoside and nonamannoside, identical regions of CV-N were perturbed. This result indicated that a linear structural expression of α1→2-linked mannoses, which is present in these two oligosaccharides, is a critical determinant for binding. Additionally, NMR relaxation measurements of the nonamannoside–CV-N complex showed the presence of two protein molecules bound per one or two molecules of oligosaccharide. The basis for this phenomenon was more thoroughly realized during the calorimetric binding studies of CV-N and the mannosides.

Calorimetric binding studies yielded a clear thermodynamic model for the interactions between CV-N and the mannosides. In these studies, CV-N binding to the chitobiose or to the core trimannoside substructure of Man-9 was not detected. However, binding to the α1→2-linked linear trimannoside, hexamannoside, or nonamannoside was readily observed, and all three sugars mediated relatively strong binding with CV-N (micromolar to submicromolar kilodalton values). Interestingly, in the nonamannoside interaction, the enthalpic contributions to the binding free energy were twice that of the trimannoside–CV-N interaction, although both sugars perturbed identical protein regions in CV-N. This result further supported earlier NMR relaxation experiments and more conclusively demonstrated that the nonamannoside mediates both multisite and multivalent binding with CV-N, cross-linking two protein molecules ostensibly via its α1→2-linked mannose arms. The stoichiometry was calculated as an overall 1:1 binding between nonamannoside and CV-N. A mechanism for the practically irreversible association of CV-N with Man-9 brought about by these unique multivalent interactions can thus be readily discerned. The potent HIV-inactivating activity of CV-N may be due in large part to its ability to cross-link discrete oligomannose-containing regions of gp120 and blocking gp120 from participating in virus-cell fusion (Figure 1).

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Getting a "Grip" on Reverse Transcription: Fidelity of DNA Synthesis

This is the final in a series of three articles focusing on the role of RNase H primer grip region in HIV reverse transcription.

Zhang W-H, Svarovskaia ES, Barr R, and Pathak VK. Y586F mutation in murine leukemia virus reverse transcriptase decreases fidelity of DNA synthesis in regions associated with adenine-thymine tracts. *Proc Natl Acad Sci U S A* 99: 20090-5, 2002.

Genetic variation in retroviral populations provides a mechanism for human immunodeficiency virus type 1 (HIV-1; the virus that causes AIDS) and other retroviruses to escape host immune responses and develop resistance to all known antiretroviral drugs. Polymerization errors during DNA synthesis by reverse transcriptase (RT) are a major source of genetic variation in retroviral populations. In the last few years, structural determinants of murine leukemia virus (MLV) and HIV-1 RTs that are important for fidelity of DNA synthesis *in vivo* have begun to be identified.

RTs contain a DNA polymerase domain that copies RNA or DNA to form viral DNA that is integrated into the host cell chromosome to form a provirus. In addition, RTs contain a ribonuclease (RNase) H domain that degrades RNA in an RNA:DNA hybrid. Both enzymatic activities are essential for viral DNA synthesis and replication. The RNase H primer grip domain was recently identified as a set of amino acids in the HIV-1 RT that contacts the DNA primer in a template-primer duplex; this domain was hypothesized to position the DNA primer strand near the RNase H active site and aid in RNase H activity and cleavage specificity (Sarafianos SG, et al., *EMBO J* 20: 1449-61, 2001). Based on sequence alignments, the Y501 residue of HIV-1 RT, which is a component of the HIV-1 RNase H primer grip (and its equivalent the Y586 residue of MLV RT), is part of a conserved DSXY motif that is present in most retroviral RNase H domains.

Recently, our laboratory determined the effect of the MLV RT Y586F mutation on the fidelity of viral DNA synthesis *in vivo*. The presence of the Y586F substitution was associated with a 5.4-fold and 4.3-fold increase in the mutation rates of the *lacZ* and green fluorescent protein (GFP) reporter genes, respectively—the largest increases reported to date. Analysis of substitution mutations induced in GFP reporters indicated that a large proportion of the substitutions induced by the Y586F mutation were clustered near adenine-thymine tracts (AAAA, TTTT, and AATT; also known as A-tracts), which are known to induce bends in DNA. The A-tracts were within 18 nucleotides of a high proportion of the substitutions induced by the Y586F mutation, representing a 17.2-fold increase compared with the wild-type RT. These results indicated that the Y586F mutation increases the frequency of polymerization errors in the vicinity of template-primer duplexes with unusual conformations.

Because the A-tract sequences are associated with bends in DNA, the conformation of the template-primer complex appears to be a significant structural determinant of fidelity. The results suggest that the wild-type RT evolved to facilitate a proper conformation of the template-primer that is amenable to incorporation of the correct nucleotides at the polymerase active site. When wild-type RT encounters irregular template-primer conformations such as those induced by the presence of A-tracts, certain structural determinants of RT facilitate an alteration of the template-primer conformation that is necessary for high Y586 residue, and the RNase H primer grip region appears to be a structural determinant of RT that is important for inducing a conformation of the template-primer duplex that is necessary for accuracy of DNA synthesis. The structures of the RNA:DNA and DNA:DNA hybrids in complex with HIV-1 RT indicate that both hybrids possess A-form structure near the polymerase active site, a 41° bend, followed by B-form DNA near the RNase H active site (Sarafianos SG, et al., *EMBO J* 20: 1449-61, 2001). Based on comparison with other DNA polymerases, this template-primer conformation, especially the A-form conformation of the template-primer near the active site, is believed to contribute to fidelity by reducing the impact of sequence-dependent structural alterations on fidelity.

These results have identified two features of the reverse transcription complex that are important for accuracy of DNA synthesis. First, the A-form template-primer conformation near the polymerase active site appears to be an important feature of the polymerase active site that may be critical for accuracy of DNA synthesis. Second, the Y586 residue and the MLV RNase H primer grip can affect the conformation of the template-primer near the polymerase active site as well as the RNase H active site. The RNase H primer grip appears to be an important structural element that is critical for maintaining a proper template-primer conformation near the polymerase active site, even when irregular template-primer conformations such as A-tracts are encountered.

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Real-time PCR Technology

The NCI/SAIC Gene Expression Laboratory (GEL) in Frederick assists NCI investigators with gene expression tasks, including mRNA isolation and purification from a variety of biological specimens, and characterization and quantification of nucleic acids using real-time PCR technology. The Laboratory can also generate and purify recombinant adenoviruses for gene expression studies. The GEL is part of the NCI/SAIC Research Technology Program (http://web.ncifcrf.gov/rtp/) and is directed by Narayan K. Bhat, Ph.D.

The GEL's Real-Time PCR Core has been in operation since September 2001 and offers technical expertise and support services to quantify nucleic acids in absolute or relative levels. Quantification is accomplished by monitoring the PCR amplification of nucleic acids in real time (i.e., during the process) via fluorescent detection rather than at the end of the process. Nucleic acid fluorescence is monitored using labeled hybridization probes (TaqMan or molecular beacons), labeled PCR primer (Amplifluor), or DNA-binding dye SYBR Green. The GEL has two Applied Biosystems PRISM 7900HT sequence detection systems with 96- and 384-well plate capability, an automatic plate-loading robotic system, and a Biomek FX laboratory workstation. With 384-well formats, the GEL can carry out approximately 3,000 real-time PCR reactions and more than 10,000 end-point PCR reactions in 8 hours. Real-time PCR instruments can be used both for gene expression quantitation and the detection of single-nucleotide polymorphisms (SNPs) using the fluorogenic 5' nuclease assay. An example of a real-time PCR assay for quantitating glyceraldehyde-3-phosphate dehydrogenase mRNA is shown in Figure 1.

The technical expertise offered by the Real-Time PCR Core includes total or cytoplasmic RNA and polyA RNA preparation, cDNA synthesis, training, consultation for real-time PCR assay design, establishment of dual-labeled probes and endogenous controls set up, run assays, and data analysis. The GEL has set up and run real-time PCR assays (relative and absolute) for a variety of genes associated with cancer development and progression and for many housekeeping genes (including glyceraldehyde-3-phosphate dehydrogenase and beta-actin) using TaqMan, Ampliflour, or SYBR Green dye probes. In collaboration with the Laboratory of Molecular Technology (NCI/SAIC Research Technology Program), the GEL has established a real-time PCR-based assay for SNP genotyping of genes associated with cancer; this expertise is now available to NCI investigators. The GEL has also established a database resource from the published literature for these assays.

In consultation with Drs. David Goldstein (Office of the Director, CCR), Robert Strausberg (Cancer Genomics Office), and Robert Clifford and Kenneth Buetow (Laboratory of Population Genetics), the GEL has developed the Quantitative PCR Primer Database (QPPD). This database (http://web.ncifcrf.gov/rtp/gel/primerdb) provides information about primers and probes that can be used to quantitate human and mouse mRNA by reverse transcription PCR assays. All data are gathered from published articles cited in PubMed. The QPPD can be searched to find primer sets and assay type for a gene of interest. Search results will provide primer sets and probes for a given gene, primer location, amplicon size, assay type, positions of SNPs, literature references, available I.M.A.G.E. cDNA clones, the primer viewer, and a graphical representation of the gene and primer sets, which includes hyperlinks to gene information from the Cancer Gene Anatomy Project (http://www.ncbi.nlm.nih.gov/ncicgap/) and the CGAP SNP viewer (http://gai.nci.nih.gov/cgi-bin/GeneViewer.cgi). The primer viewer also provides information regarding the number of times a particular primer set has been used and links to purchase I.M.A.G.E. cDNA clones to be used as a positive control in real-time PCR assays. Investigators may submit newly published primer and probe information for inclusion into the QPPD. Online primer submission is available and is subject to review by Gene Expression Laboratory staff. Currently, the QPPD contains more than 1,000 curated records.

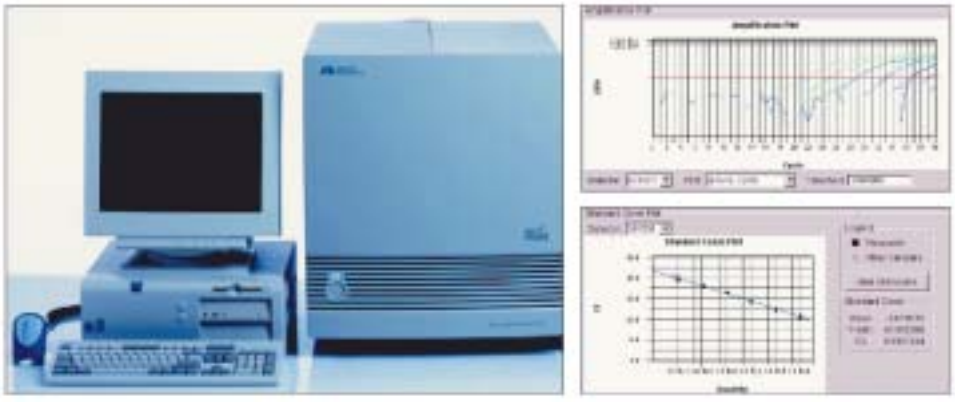

Figure 1. Human glyceraldehyde-3-phosphate dehydrogenase (GAPD) plasmid DNA (10 to 1,000,000 copies) was amplified by PCR using the TaqMan FAM/TAMRA probe. *Left:* FAM fluorescence was monitored continuously using the Applied Biosystems PRISM 7900HT sequence detection system. *Right:* The threshold cycle (Ct) for each dilution was plotted for log[GAPD]. Up to 10 copies of GAPD can be detected easily.

The GEL's Adenovirus Production Group offers technical expertise and support services to generate recombinant adenoviruses (starting from the expression vector) and to purify adenoviruses in large quantities. Areas include training; consultation in adenovirus vector engineering; and recombinant adenovirus generation, amplification, purification, and titer determination. This Group also provides consultation in the expression of genes in target cells using adenoviruses.

To learn more about the resources available at the GEL, visit http://web.ncifcrf.gov/rtp/labs/GEL/default.asp or contact Dr. Bhat.

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■ ON THE TENURE TRACK

Yikang Rong, Ph.D.

Yikang Rong, Ph.D., joined CCR's Laboratory of Molecular Cell Biology in January 2002 as a tenure-track principal investigator. Born in the People's Republic of China, he graduated from the University of Science and Technology of China in 1990, majoring in cell biology, and from the University of Utah with a Ph.D. in genetics in 1998. While at the University of Utah, Dr. Rong studied eye pigmentation in the fruit fly *Drosophila melanogaster* in Dr. Kent Golic's lab. Trained as a classical geneticist, Dr. Rong fully understood the importance of mutants to *in vivo* studies of gene functions. At that time geneticists were not able to perform targeted knockout by homologous recombination in *Drosophila*, even though such a powerful tool had allowed their colleagues in yeast and mouse research to modify essentially any gene at will for more than 20 years. As a graduate student, Dr. Rong and his advisor Dr. Golic started to develop a novel scheme to accomplish gene targeting, which prompted Dr. Rong to stay on as a postdoctoral fellow and continue his quest for a targeted knockout method for *Drosophila*. The breakthrough came on Christmas day of 1999, when the first "targeted" fly was discovered. Since then scientists worldwide have used this method to knock out genes of interest in *Drosophila*.

As he was developing the knockout method, Dr. Rong also developed a way to generate site-specific DNA double-strand breaks (DSBs) in *Drosophila* chromosomes. The repair of these DSBs is currently the focus of Dr. Rong's group at CCR. The group will study the repair process from a developmental perspective in a multicellular organism, which is still a young field in current DSB repair studies. Dr. Rong will focus on identifying new features of DSB repair that are specific to higher eukaryotes, which may contribute to our understanding of how defects in DSB repair often lead to cancer in humans.


Dr. Rong

Dr. Rong's wife, Debbie Wei, also works as a researcher at CCR. They have a 4-month-old daughter and a 3-year-old son. They love the outdoors, but now their spare time is taken up by pampering their babies.

■ ADMINISTRATIVE LINKS

Look to the "Stars" for Recruitment

StarGazer is a new databank that enables NCI science and administrative managers to conveniently access a growing pool of highly qualified candidates. Candidate searches can be conducted by job category, scientific discipline, administrative specialty, and recruitment event source code. Searches can be further narrowed by date-range and locality. Applicants are recruited from job fairs, scientific society meetings, college recruitment events, advertisements, general public interest, and NCI internal referrals; currently more than 1,500 candidates are available. StarGazer is restricted for use by NCI hiring managers (managers, supervisors, team leaders, and individuals designated by their director as a recruitment point of contact). Entry to StarGazer is gained through a designated access code. To request access, hiring managers should contact Keith Ariola at ariolak@mail.nih.gov or by phone at 301-402-0306.

Lobbying the U.S. Congress

Occasionally, NIH personnel receive Email messages through the NIH system from scientific associations soliciting participation in campaigns to lobby the U.S. Congress in regard to specific legislation or issues involving biomedical research. While nothing is wrong with receiving such messages, please note that the Anti-lobbying Act prohibits the use of appropriated funds to lobby members of Congress regarding any legislative matter. Using official time or resources to make such contacts outside of official channels, in relation to any legislative matter, is prohibited.

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